1083-8791/05/1105-0004$30.00/0
doi:10.1016/j.bbmt.2005.02.003

Rapid and Complete Donor Chimerism after Unrelated Mismatched Cord Blood Transplantation in 5 Children with β-Thalassemia Major

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Received December 7, 2004; accepted February 5, 2005

ABSTRACT

Hematopoietic stem cell transplantation is currently the only curative therapy for β-thalassemia major. However, <30% of patients have unaffected HLA-identical siblings to serve as donors. We investigated the feasibility of using umbilical cord blood transplants from unrelated HLA mismatched donors and a myeloablative preparative regimen that did not involve total body irradiation. Between October 2003 and November 2004, 5 children with β-thalassemia major received busulfan, cyclophosphamide, and antithymocyte globulin before cord blood transplantation (median dose, 8.8 × 10^7 cells per kilogram of body weight) from unrelated donors (1 or 2 of 6 HLA antigens were mismatched) and were then evaluated for engraftment, adverse effects, and treatment outcome. The median times to neutrophil engraftment, red blood cell transfusion independence, and platelet engraftment were 12, 34, and 46 days after transplantation, respectively. All patients showed grade II or III acute graft-versus-host disease; none developed extensive chronic graft-versus-host disease until the date of last contact. All patients were alive at a median follow-up of 303 days after transplantation, with complete donor chimerism and transfusion independence. These results are encouraging and clearly show the feasibility of unrelated mismatched umbilical cord blood transplantation in the treatment of children with β-thalassemia major.

KEY WORDS

Umbilical cord blood transplantation • Unrelated donor • β-Thalassemia major • HLA mismatch

INTRODUCTION

The current conventional treatment of β-thalassemia major consists of lifelong monthly blood transfusion combined with daily subcutaneous iron chelation therapy with desferrioxamine from approximately 2 years of age onward [1,2]. The use of umbilical cord blood (UCB) offers a potential means of alleviating the shortage of donors that has plagued bone marrow transplantation since its inception [3-5]. Extending the use of UCB to unrelated mismatched donor transplants would greatly facilitate the identification and procurement of the pluripotent stem cells required for hematopoietic stem cell transplantation (HSCT) for large numbers of patients for whom no acceptable family donor is available.

MATERIALS AND METHODS

Patients

Five consecutive patients with β-thalassemia major underwent transplantation with a UCB graft at Chang Gung Children's Hospital between October 2003 and August 2004. The diagnosis of each β-thalassemia major participant was confirmed by DNA sequencing. These patients were enrolled onto this study because they had neither an HLA-identical

Table 1. *Main Clinical and Biologic Characteristics of the 5 Patients*

Variable	Patient				
	1	2	3	4	5*
Clinical					
Age (y)	3.7	2.3	3.6	5.8	11.4
Genotype	IVS II-654 and p28	Homozygous IVS 654	Homozygous IVS 654	IVS II-654 and codon 43	IVS II-654 and codon 41/42
Disease status	Lucarelli class I	Lucarelli class I	Lucarelli class I	Lucarelli class I	Lucarelli class I
Pretransplantation serum ferritin level (μg/L)	515	1583	2461	797	2125
HLA type					
Patient	A2, A24, B46, B48, DRB1 1312, 1501	A11, —, B4001, B46 DRB1 0406, 1501	A0203, A2402, B1501, B3802, DRB1 0406, 1602	A0207, A2601, B1301, B4601, DRB1 1202, —	A0201, A2402, B1525, B5801, DRB1 0301, 1405
Donor	A2, A24, B46, B48 DRB1 0403, 1501	A1101, —, B4001, — DRB1 0405, 1501	A0203, —, B3802, — DRB1 0403, 1602	A0206, A1101, B1301, B4601, DRB1 1202, —	A0207, A3303, (A0201, —) B5801, —, (5601, 5801) DRB1 0301, 1405 (0301, 1401)
UCB					
Nucleated cell dose ($\times 10^7$/kg)	8.78	11.83	9.03	4.15	3.25
CD34 cell dose ($\times 10^5$/kg)	2.48	2.43	3.75	2.97	2.31

IVS indicates intervening sequence.
*Double cord blood transplantation.

related donor nor a related donor with 2 HLA mismatches, and an HLA-matched, unrelated bone marrow donor could not be located within 6 months. The median age at transplantation was 3.7 years (range, 2.3-11.4 years). This study was approved by both the Institutional Review Board at Chang Gung Memorial Hospital and the Department of Health of Taiwan.

Cord Blood Selection and Characteristics

The compatibility of HLA-A, -B, and -DRB1 was assessed by a high-resolution polymerase chain reaction technique with sequence-specific primers. Cord blood units were selected on the basis of HLA compatibility (a minimum of 4 HLA antigens shared with the recipient was required, and only 1 HLA-DRB1 single-locus mismatch was allowed). The selected cord blood had to contain, per kilogram of the recipient's body weight, at least 2×10^7 nucleated cells and 1.7×10^5 $CD34^+$ cells (determined at the time of cryopreservation). Searches for unrelated cord blood donors were processed through the StemCyte Cord Blood Bank, where 12 000 cord blood units are available locally in Taiwan.

Conditioning Regimen and Transplantation Procedure

Before the UCB transplantation procedure, all patients were placed in a high-efficiency particulate air–filtered room in the bone marrow transplantation unit. The preparative regimens consisted of oral busulfan 3.5 mg/kg/d (day −9 to −6), intravenous cyclophosphamide 50 mg/kg/d (day −5 to −2), and antithymocyte globulin (Pharmacia-Upjohn, Peapack, NJ) 30 mg/kg/d (day −4 to −1). Patients received phenytoin for prophylaxis against seizures during treatment. Mesna 50 mg/kg was administered intravenously on the days of cyclophosphamide infusion. Graft-versus-host disease prophylaxis comprised cyclosporine (2.5 mg/kg intravenously every 8 hours) from day −3 with a course of methylprednisolone (1 mg/kg intravenously every 12 hours on days 5 to 19, decreasing 25% thereafter every other day). The cyclosporine dose may be tapered beginning at least 60 days after demonstration of engraftment and full donor chimerism by short tandem repeat (STR) analysis.

In this study, 4 patients received 1 unit of cord blood, whereas 1 patient received 2 units. However, the DNA of only 1 of the 2 donors was detectable after engraftment. The numbers of infused nucleated and progenitor cells are shown in Table 1. The UCB units were thawed in a 37°C waterbath with gentle agitation and without further processing before infusion into the patients. Granulocyte colony-stimulating factor (filgrastim; Kirin, Gunma, Japan) 10 μg/kg/d was given intravenously on day 1 after transplantation and on each day thereafter until the neutrophil count remained $>1.0 \times 10^9$/L for 3 consecutive days.

Supportive Care and Posttransplantation Follow-up

Blood components were given whenever indicated to maintain hemoglobin and platelet values >8 g/dL and 20 × 10^9/L, respectively. For streptococcal prophylaxis, intravenous cefazolin was given until the neutrophil counts exceeded 0.5 × 10^9/L, and oral itraconazole (antifungal prophylaxis) 3 mg/kg daily was prescribed for the month preceding transplantation. Intravenous or oral acyclovir and oral co-trimoxazole were given to prevent cytomegalovirus (CMV) reactivation and *Pneumocystis carinii* infection. Both acyclovir and co-trimoxazole were continued as prophylaxis until day 180 after transplantation and may be continued until T-cell function is restored. Parenteral nutrition was provided for the duration of anorexia. Intravenous immunoglobulin (500 mg/kg) was given at day −6, +7, +21, +35, +56, +77, and +98 after UCB transplantation. The standard CMV pp65 antigenemia assay was performed in parallel. The positive results were quantified by counting the number of pp65-expressing cells per 50 000 leukocytes on the slide.

Myeloid engraftment was defined as 3 consecutive days of an absolute neutrophil count of ≥0.5 × 10^9/L. The last day of red blood cell (RBC) transfusion was recorded as a day of RBC transfusion independence. Platelet engraftment was defined as 7 consecutive days of a platelet count ≥50 × 10^9/L maintained without transfusion. Current methods for measuring hematopoietic chimerism are based on STR polymorphisms that distinguish recipient from donor. Serial STR polymerase chain reaction confirmed the conversion from mixed chimerism to a predominantly donor profile on day +60, +90, +120, +180, +270, and +360, followed by quarterly for the second year after transplantation and yearly thereafter. In the subset of patients with heavy iron overload who achieved myeloid engraftment, desferrioxamine was administered at 40 mg/kg/d as a 24-hour intravenous infusion to accelerate the clearance of body iron deposits.

RESULTS

Engraftment and Immune Reconstitution

High-resolution molecular typing demonstrated that 2 recipient/donor pairs had HLA 2-loci mismatches, and the remaining 3 pairs had a mismatch at 1 locus. However, neither delayed myeloid engraftment nor an increased incidence of early transplant complications was observed. Neutrophil engraftment occurred at a median of 12 days (range, 12-17 days) after transplantation. The median day to RBC transfusion independence was 34 days (range, 22-45 days). The median number of days to achieve a platelet count of >20 × 10^9/L was 46 days (range, 43-55 days; Table 2). Serum immunoglobulin levels were in the reference range 6 months after transplantation.

In the fifth patient, double cord blood units were used to minimize the risk of developing graft failure in this multiply transfused patient, who weighed >30 kg. However, the graft was derived from a female donor with a higher cell dose and better matching. All patients with myeloid engraftment showed complete donor chimerism by day +17 at the time of first STR DNA analysis. These data support the notion that certain HLA differences do not affect the clinical outcome of UCB transplantations and confirm the potential benefit of using unrelated donor UCB for β-thalassemia major.

Transplantation-Related Events

All patients developed acute graft-versus-host disease. Asymptomatic CMV reactivation was detected in

Table 2. *Characteristics of Engraftment, GVHD Grading, Outcome, and Chimerism*

	Patient				
Variable	**1**	**2**	**3**	**4**	**5***
Days until					
ANC >0.5 × 10^9/L	**17**	**12**	**14**	**12**	**12**
RBC transfusion independence	**34**	**37**	**27**	**45**	**22**
Platelets >20 × 10^9/L	**49**	**46**	**43**	**43**	**55**
GVHD grade	**I**	**II**	**I**	**II**	**III**
Outcome	**Transfusion independence**	**Transfusion independence**	**Transfusion independence**	**Transfusion independence**	**Transfusion independence**
Days after transplantation	**454**	**344**	**303**	**245**	**152**
Day of the last chimerism	**360**	**270**	**270**	**180**	**120**
Chimerism analysis (% donor cells)	**100**	**100**	**100**	**100**	**100**

ANC indicates absolute neutrophil count; RBC, red blood cell.

*Double cord blood transplantation.

2 patients (patients 2 and 5) on posttransplantation days 27 and 62, respectively. They were successfully treated preemptively with intravenous ganciclovir. Patient 2 experienced 2 episodes of reactivation during follow-up, and the disease status remained quiescent after cyclosporine was stopped 7 months after transplantation. Both patients are still taking oral acyclovir prophylactically at the time of this report. According to the criteria outlined previously, continuous chelation therapy was administered with desferrioxamine in only 1 patient (patient 5) during the early posttransplantation period.

Average Cost per Admission

The per-patient medical expense for UCB transplantation is estimated to be approximately US $40 000 plus the cost of the cord blood (which was waived for our patients).

DISCUSSION

β-Thalassemia major often causes transfusion-related complications, which have a major economic and even political impact. Banked, unrelated, partially HLA-matched UCB is an alternative stem cell source for patients in need of transplantation therapy who lack traditionally matched donors. Full HLA compatibility is desirable in HSCT, but mismatches of 1, 2, or 3 antigens are acceptable with UCB [6]. Because several clinical studies have demonstrated improved survival after infusion of higher cell doses per kilogram of body weight, cord blood units containing high cell doses were chosen for our study [7-9].

Traditional techniques for HSCT rely on the production of biologic "space" in the recipient's bone marrow compartment by myeloablative conditioning. A major disadvantage to myeloablative conditioning is a transient period of myelosuppression before bone marrow reconstitution, during which the bone marrow transplant recipient is susceptible to infection. Engraftment usually seems slower after transplantation of cord blood than marrow or peripheral blood [4,10,11]. A consensus is emerging that UCB grafts of higher cell doses should be selected wherever possible to optimize engraftment [12]. It is noteworthy that the UCB we used in this study (which had a higher number of CD34 cells or total nucleated cells) led to earlier neutrophil recovery. We attribute these good results to the fact that all the thalassemia patients included in the study were classified as Lucarelli class I. The use of a higher dose of growth factor (10 μg/kg/d) may also have contributed to speedy engraftment. UCB contains enough hematopoietic stem cells to reconstitute bone marrow in children. Moreover, Wagner et al. [7] suggested that a higher CD34 cell dose partially overcomes the negative effect of UCB with ≤2 HLA disparities.

Iron overload is frequent in patients with β-thalassemia major and after UCB transplantation; it is often caused by ineffective erythropoiesis, intestinal hyperabsorption, and RBC transfusions. Relatively little is known about the reconstitution of hematopoiesis in thalassemia after transplantation. In our study, the follow-up electrophoresis in the first patient identified only small amounts of hemoglobin F (16.1% and 1.6%) 6 and 12 months after transplantation, respectively. HSCT acts as a double-edged sword. Some thalassemia patients may develop either mixed chimerism or late rejection 1 year after transplantation, but others may die prematurely or experience delayed recovery [13,14]. The success of UCB transplantation in our study strongly suggested that ideal candidates for HSCT are young patients without underlying complications of their disease or transfusional iron overload.

HSCT with donors other than HLA-identical siblings is associated with high morbidity and poor survival [15]. UCB transplantation seems to have a higher risk of nonengraftment and secondary rejection [16-18]. A more intensive conditioning regimen might prevent early graft failure [19]. Our conditioning regimens have been generally well tolerated, and all patients showed myeloid engraftment by day +17 after transplantation. All patients had sustained engraftment. Our result confirmed previous findings on the association of nucleated cell dose with the speed and probability of engraftment [20]. In addition, this study presents recommendations to policy makers for integrating unrelated mismatched UCB transplantation into health promotion initiatives.

The transplantation of unrelated mismatched UCB is highly valuable for patients with β-thalassemia major who have no HLA-suitable sibling donor, and it is clearly cost-effective when compared with conventional treatment with blood transfusions and iron chelation therapy. In summary, our results demonstrate the importance of the graft cell dose in accelerating engraftment of UCB transplants, even in recipients who receive grafts disparate for 2 HLA loci.

ACKNOWLEDGMENTS

This study was supported by grants from Chang Gung Memorial Hospital (CMRP G33020) and was accomplished thanks to the efforts of Professor Delon Wu. The work was scientifically supported by the HLA Laboratories of StemCyte, Inc.

REFERENCES

1. Cao A, Galanello R, Rosatelli MC, et al. Clinical experience of management of thalassemia: the Sardinian experience. *Semin Hematol.* 1996;33:66-75.

2. Karnon J, Zeuner D, Brown J, et al. Lifetime treatment costs of β-thalassemia major. *Clin Lab Haematol.* 1999;21:377-385.
3. Fasouliotis SJ, Schenker JG. Human umbilical cord blood banking and transplantation: a state of the art. *Eur J Obstet Gynecol Reprod Biol.* 2000;90:13-25.
4. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998;339:1565-1577.
5. Sanz MA. Cord-blood transplantation in patients with leukemia—a real alternative for adults. *N Engl J Med.* 2004;351: 2328-2330.
6. Wagner JE, Rosenthal J, Sweetan R, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. *Blood.* 1996;88:795-802.
7. Wagner JE, Barker JN, DeFor TE, et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood.* 2002;100:1611-1618.
8. Locatelli F, Rocha V, Chastang C, et al. Factors associated with outcome after cord blood transplantation in children with acute leukemia. *Blood.* 1999;93:3662-3671.
9. Ballen KK, Wilson M, Wuu J, et al. Bigger is better: predictors of hematopoietic potential of umbilical cord blood units. *Bone Marrow Transplant.* 2001;27:7-14.
10. Wadlow RC, Porter DL. Umbilical cord blood transplantation: where do we stand? *Biol Blood Marrow Transplant.* 2002;8:637-647.
11. Ballen K, Broxmeyer HE, McCullough J, et al. Current status of cord blood banking and transplantation in the United States and Europe. *Biol Blood Marrow Transplant.* 2001;7:636-645.
12. Grewal SS, Barker JN, Davies SM, Wagner JE. Unrelated donor hematopoietic cell transplantation: marrow or umbilical cord blood? *Blood.* 2003;101:4233-4244.
13. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. *N Engl J Med.* 1990;322:417-420.
14. Sullivan KM, Parkman R, Walters MC. Transplantation for non-malignant disease. *Hematology (Am Soc Hematol Educ Program).* 2000;319-338.
15. Grewal SS, Kahn JP, MacMillan ML, et al. Successful hematopoietic stem cell transplantation for Fanconi anemia from an unaffected HLA-genotype-identical sibling selected using preimplantation genetic diagnosis. *Blood.* 2004;103:1147-1151.
16. Fang J, Huang S, Chen C, et al. Umbilical cord blood transplantation in Chinese children with beta-thalassemia. *J Pediatr Hematol Oncol.* 2004;26:185-189.
17. Cohen Y, Nagler A. Hematopoietic stem-cell transplantation using umbilical-cord blood. *Leuk Lymphoma.* 2003;44:1287-1299.
18. Sanz GF, Saavedra S, Planelles D, et al. Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies. *Blood.* 2001;98:2332-2338.
19. Ball LM, Lankester AC, Giordano PC, et al. Paediatric allogeneic bone marrow transplantation for homozygous beta-thalassaemia, the Dutch experience. *Bone Marrow Transplant.* 2003; 31:1081-1087.
20. Migliaccio AR, Adamson JW, Stevens CE, et al. Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. *Blood.* 2000;96:2717-2722.